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14. ABSTRACT

We investigated effects of biofilms and surface texture on settlement of larvae onto surfaces in realistic water flow characteristic of harbors, where the colonization of ships by fouling organisms takes place. We found that flow along surfaces in Pearl Harbor, HI, was oscillatory due to wind chop and ship wakes. Flume experiments showed such oscillations altered the effects of surface rugosity on the shear stresses imposed on settling larvae. Larvae of biofouling tube worms and bryozoans change their behavior when they contact biofilmed surfaces from swimming in straight paths to circling and crawling, but do not respond to dissolved chemicals from biofilmed surfaces. Newly-settled larvae of tube worms, barnacles, and bryozoans, and juvenile tube worms, adhere more tightly to biofilmed surfaces than to clean ones.

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Final Report

GRANT #: N00014-03-1-0078

PRINCIPAL INVESTIGATOR: Dr. Michael G. Hadfield

GRANT TITLE: The effects of surface texture, flow, and dissolved cues from biofilms on settlement and attachment of fouling organisms to marine coatings.

AWARD PERIOD: 1 October 2002 - 31 December 2005

OBJECTIVES: Our objective was to investigate the effects that natural and man-made coatings have on the settlement and adhesion of the larvae of fouling organisms onto surfaces in realistic water-flow conditions characteristic of harbors. Our specific objectives were to: **1.** Supply larvae for experiments from target species representing different phyla, swimming capabilities, and attachment mechanisms; **2.** Measure water velocity profiles and turbulence near submerged surfaces in harbors; **3.** Determine which target species have larvae that alter their locomotion in response to dissolved substances from surfaces (e.g., biofilms and coatings) (this objective was expanded to reflect discovery that larval species tested so far respond only to surface-bound cues); **4.** Assess the dispersal and dilution of dissolved cues released from surfaces in harbors using larval bioassays; **5.** In laboratory simulations of small-scale flow near surfaces in harbors, quantify the effects of different coatings on the trajectories of and encounters with those surfaces by larvae of the target species; **6.** Measure effects of coatings (e. g., biofilms, fouling organisms, man-made coatings) on attachment strengths of larvae of the target species; **7.** Test feasibility of using laser-Doppler velocimetry in a large wave/flume to determine stresses encountered by and probability of attachment of microscopic larvae settling on surfaces with different degrees of fouling or textures.

APPROACHES: We used different approaches to accomplish each objective. **1.** To obtain a reliable supply of larvae for experimentation, potential target species were brought into the laboratory throughout the year to determine spawning time and establish culturing techniques. **2.** Water velocities were measured under a variety of ambient flow conditions at positions 2, 4, 8, 16, and 32 cm from biofouled surfaces on a floating raft in Pearl Harbor, HI, using an electromagnetic flow meter. Spectral analyses of these data were conducted to characterize the frequency distribution of velocity fluctuations in this harbor flow. Video records of the dispersion of dye released near floating surfaces in Pearl Harbor were analyzed to quantify advective transport and turbulent mixing (of chemical cues from coatings; of larvae). **3.** Videomicrography was employed to quantify the swimming and crawling behavior of larvae over biofilmed or clean test surfaces in the laboratory. Behavior when larvae could contact surfaces was compared with behavior when a permeable Nitex screen prevented contact but allowed dissolved substances released from test surfaces to contact larvae **4.** Swimming behavior of larvae of the fouling tube worm *Hydroides elegans* was videotaped in water samples collected at various distances from biofouled surfaces in Pearl Harbor to determine if dissolved cues from fouling organisms in natural waters affected larval behavior. **5.** A miniature wave/flow tank was designed, built, and tested, in which the behavior of microscopic larvae near different types of surfaces can be videotaped in oscillatory flow mimicking harbor conditions. **6.** The resistance of one or more post-settlement stages of three species (tube worm, bryozoan, barnacle) to removal from test surfaces by water flow was determined in a turbulent channel-flow apparatus in which the percent of the animals that were removed by known shear stresses from biofilmed vs. clean test surfaces was determined. **7.** We placed panels

bearing fouling communities of different stages collected in Pearl Harbor in turbulent, wavy flow in a large flume and used laser-doppler anemometry to measure flow rates about 500 μm from surfaces.

ACCOMPLISHMENTS: Water flow in harbors determines the transport of larvae to ship surfaces, the hydrodynamic forces tending to wash larvae off surfaces, and the dispersion of chemical cues from surfaces. Our water-velocity measurements near floating surfaces in Pearl Harbor showed that, although mean water flow is slow (10 - 20 cm/s), the velocity fluctuates greatly. In addition to net current flow, surfaces in harbors are exposed to oscillatory flow due to small wind-driven waves and to the wakes of boats and ships moving through the harbor. In ship wakes, instantaneous velocities can be quite high (~60cm/s). Our analysis of dye dispersion showed that as water sloshes back and forth relative to floating surfaces, the net transport of material in the water (such as larvae or dissolved substances released from surface coatings) is slow (0.04 m/s, s.d. = 0.02, n = 11 dye studies). Furthermore, our dye studies showed that substances released from coatings are spread in the water along surfaces via shear dispersion at twice the rate (0.0004 m^2/s, s.d. = 0.0002) that they dispersed away from surfaces (0.0002 m^2/s, s.d. = 0.0001).

We successfully reared larvae of four target species of fouling animals for use in our experiments. The species were chosen to represent strong (muscular) and weak (ciliary) swimmers with varying attachment modes: cyprid larvae of the barnacle *Balanus amphitrite*, a strong muscular swimmer that attaches by secreting a strong cement from its antennules; tadpole larva of an ascidian, *Didemnum* sp., a weak muscular swimmer that attaches by soft secretions from papillae; nectochaete larva of the tube worm *Hydroides elegans*, a weak ciliary swimmer that originally attaches by secreting a membranous tube around itself and subsequently secreting a calcareous tube attached to the substratum; coronate larva of the bryozoan *Bugula neritina*, a weak ciliary swimmer that adheres by eversion of a sticky section of its soft body. We used these target species to study effects of: (1) dissolved chemical cues from surfaces on larval swimming; (2) contact with surfaces on larval behavior; and (3) coatings on adhesion by newly-settled larvae.

We found differences in larval behavior during contact with clean substrata versus biofilmed surfaces. Videomicrography of larvae of both *H. elegans* and *B. neritina* revealed that most larvae swam near clean surfaces, whereas most larvae stopped swimming and crawled on biofilmed surfaces. Furthermore, the larvae that did swim near biofilmed substrata turned and circled, whereas larvae near clean surfaces swam along straight paths. These behavioral effects did not occur if the larvae were separated from the biofilm by a 20-micron Nitex mesh that prevented larval contact with the biofilm, but did not prevent the passage of dissolved chemicals released from the biofilmed surfaces. The latter experiments showed that there was no soluble cue from the biofilm stimulating settlement.

We measured larval adhesion to biofilmed and non-biofilmed surfaces by determining the percent of the settled larvae on a surface that remained attached after exposure to a boundary shear stress of 75 Pa for 5 minutes in a turbulent channel-flow tank. We showed that larvae of *H. elegans*, *B. amphitrite*, and *B. neritina*, adhered more tightly to biofilmed than to clean surfaces. We also investigated the interaction of biofilms and substratum type. The positive influence of biofilms on adhesion was confirmed for polystyrene as well as for glass. For the tube worm, *H. elegans*, we found that adhesion of the organic primary tubes of newly-settled individuals was stronger on biofilmed than on clean surfaces. We also found that, for late-stage settled tubeworms that had produced calcified secondary tubes, the number of worms left

inhabiting a tube after exposure to flow was greater on surfaces when a biofilm was present.

In collaboration with colleagues at Univ. of Colorado, Koehl carried out measurements of flow velocities over panels that had been naturally fouled in Pearl Harbor during studies carried out by Hadfield's research group. The study included panels at early stages of biofouling, bearing only scattered worm tubes, and at late stages of fouling, with dense assemblages of barnacles, oysters, ascidians and sponges. Using a focused laser-doppler anemometer, it was possible to determine flow velocities within 500 microns of surfaces at diverse locations within the fouling communities and to determine how velocities were affected by increasing amounts of biofouling. We found that as the rugosity of the surface increased (i.e., as the fouling community developed), the range of magnitudes of flow velocities and stresses encountered by settling larvae greatly increased (both more protected and more exposed micro-habitats were available for settlement in more mature fouling communities, i.e., on rougher surfaces). When compared with unidirectional currents, the oscillatory flow associated with wind chop and with ship wakes typical of harbors had significant effects. Such waves not only changed which microhabitats within a community were exposed to the highest stresses, but also greatly decreased the probability that larvae could adhere to surfaces. In addition to the above accomplishments, a graduate student (vonDassow) associated with this project studied how water flow across surfaces affects the development and growth patterns of fouling bryozoans.

CONCLUSIONS: We found that the field-relevant flow conditions that should be used in the laboratory to study settlement of larvae onto coatings are oscillatory, and that water-borne materials (e.g., larvae; chemical cues released from surfaces) are dispersed much more rapidly along surfaces in harbor flow than they are transported away from them. Flume experiments showed such oscillations altered the effects of surface rugosity on the shear stresses imposed on settling larvae. Larvae of tube worms and bryozoans change their behavior when they contact biofilmed surfaces from swimming in relatively straight paths to circling in place and crawling. However, larvae of these species do not show such behavioral changes in response to dissolved chemicals from biofilmed surfaces. Newly-settled larvae of tube worms, barnacles, and bryozoans, and juvenile tube worms, all adhere much more tightly to biofilmed surfaces than to clean ones.

SIGNIFICANCE: Navy ships spend a large percentage of their time in harbors, where most larval settlement of fouling organisms occurs. Although laboratory studies of larval settlement and adhesion to coatings are usually done in unidirectional water currents, we found that flow in the field in harbors has an oscillatory component due to wind chop and ship wakes that can have a profound effect on the stresses on larvae settling on surfaces. Therefore, we have designed and built an oscillatory-flow tank in which to conduct our future experiments on larval settlement behavior onto various natural and man-made coatings. We also learned that once biofilms develop on different types of surfaces, larvae behave differently near those surfaces and adhere to them more tightly. Our work shows that an important approach to coating development should be to create biofilm-disrupting surfaces that thereby can lower larval attachment strength.

PATENT INFORMATION: none

PUBLICATIONS AND ABSTRACTS:

Publications in peer reviewed journals:

von Dassow, M. (2005) Effects of Ambient Flow and Injury on the Morphology of a Fluid Transport System in a Bryozoan. Biol. Bull. 208: 47-59.

von Dassow, M. (2005) Flow and conduit formation in the external fluid-transport system of a suspension feeder. J. Exp. Biol. 208: 2931-2938.

Zardus, J. D. and M. G. Hadfield. 2004. Larval development and complemental males in *Chelonibia testudinaria*, a barnacle commensal with sea turtles. Journal of Crustacean Biology 24: 409-421.

Papers submitted:

Miles, C., Hadfield, M .G. and M. L. Wayne. Estimates of heritability for egg size in the serpulid polychaete Hydroides elegans. Mar. Ecol. Prog. Ser. (submitted).

von Dassow, M. Function-dependent development in a colonial animal. Biol. Bull. (submitted).

Papers in preparation:

Crimaldi, J.P., Dombroski, D. E., and Koehl, M .A. R. Larval attachment in different microhabitats within fouling communities subjected to turbulent currents, wind chop, and ship wakes. (to be submitted to Marine Ecology Progress Series)

Hadfield, M. G., J. D. Zardus, C. Tran, Y, Huang, T. Cooper and M. A. R. Koehl. Settlement responses of larvae of biofouling invertebrates to marine biofilms. (to be submitted to Marine Biology)

Koehl, M .A. R., and T. Cooper. Water flow and mass transport near surfaces in harbors: The importance of wind and ship wakes. (to be submitted to Limnology and Oceanography)

Zardus, J. D., Y. Huang, B. Nedved and M. G. Hadfield. Bacterial biofilms enhance adhesion strength of settling invertebrate larvae. (to be submitted to Science).

Ph.D. Dissertations:

von Dassow, M. (2005) Function-dependent development in an encrusting bryozoan. Ph.D. Dissertation, University of California, Berkeley.

Abstracts/Presentation/Posters/Conference Proceedings/Lectures

Hadfield, M. G. 2003. Biofilms and larval settlement. Australian Marine Science Association, Brisbane, Australia, (invited plenary lecture).

Hadfield, M. G. 2003. Biofilms and larval settlement. Autonomous University of Baja California, Mexico (3 invited lectures).

Hadfield, M. G. 2003. Biofilms and larval settlement. Department of Biology, San Diego State University (invited lecture).

Hadfield, M. G. 2003. Larval settlement. Centennial Symposium, Friday Harbor Laboratories, University of Washington (invited lecture).

Hadfield, M. G. 2003. Biofilms and larval settlement. American Society of Microbiology, Special Symposium on Biofilms, Victoria, British Columbia, Canada (invited lecture).

Hadfield, M. G. 2003. Can we predict where larvae will settle? Can we test the prediction? International Larval Biology Conference, Hong Kong, China (invited plenary lecture).

Hadfield, M. G. 2004. Biofilms and Biofouling: bacteria dictate larval settlement *(but not all bacteria are equal)*. International Society for Microbial Ecology, Cancun, Mexico (invited lecture).

Hadfield, M. G. 2005. Why do marine invertebrate larvae settle where they do? Soluble vs. absorbed cues to settlement. National Institute of Oceanography, Goa, India. (invited lecture).

Hadfield, M. G. 2005. Marine biofilms: cues for larval settlement and sites for hidden pathogens. Hopkins Marine Station, Stanford University. (invited lecture).

Hadfield, M. G. 2005. Marine biofilms: cues for larval settlement and sites for hidden pathogens. Department of Integrative Biology, University of California, Berkeley.

Hadfield, M. G. Invited lecture, Institute of Oceanology, Autonomous University of Baja
California, "Biofilms and biofouling. Jan. 2005.

Hadfield, M. G., Koehl, M. A. R. and Zardus, J. D. (2004) The effect of chemical cues on the adhesion strength of settling invertebrate larvae. Annual Meeting of the Society for Integrative and Comparative Biology, Jan. 5 - 9, new Orleans, LA (abstract) 67.7).

Hadfield, M. G., Shikuma, N., Huang, Y., Nedved, B., Zardus, J. (2005). Do bacteria dictate the sequence of biofouling community development by signaling larval settlement? Integrative and Comparative Biology 44(6):562.

Koehl, M. A. R. 2003. Hydrodynamics of cue-induced settlement by larvae in turbulent marine environments. BIOFLOW Symposium, Rostock, Germany (invited, Keynote Speaker).

Koehl, M .A. R. 2003. How do larvae settle in the right place in a turbulent world? Symposium on Robustness in Biological and Social Systems, Santa Fe Institute (invited lecture).

Koehl, M. A. R. 2004. Hydrodynamics of larval settlement . International Sustainability Days Conference . Stanford University. (invited lecture),

Koehl, M. A. R. 2004. Hydrodynamics of larval settlement. Department of Civil, Environmental, and Architectural Engineering, University of Colorado. (invited lecture).

Koehl, M. A. R. 2004. How do marine larvae land in the right place in turbulent flow? Division of Engineering and Applied Sciences , Harvard University (invited lecture).

Koehl, M. A. R. 2004. How do marine larvae land in the right place in turbulent flow? Division of Engineering and Applied Sciences , Harvard University (invited lecture).

Koehl, M. A. R. 2005. Larval settlement in turbulent flow. H. Burr Steinbach Visiting Scholar, Woods Hole Oceanographic Institution (invited lecture).

Koehl, M. A. R. and Hadfield, M. G. 2005. Dissolved chemical cues affect retention and attachment of larvae within coral reefs. Integrative and Comparative Biology 44(6): 584.

Koehl, M. A. R., Koseff, J., Reidenbach, M., Strother, J., Crimaldi, J. Wiley, M, and Hadfield, M. 2004. How animals of different sizes

encounter chemical cues in turbulent ambient water flow. Symposium on Biological Adaptations to Turbulence . Annual meeting of American Society of Limnology and Oceanography. (invited lecture).

Shikuma, N. J. and Hadfield, M. G. 2005. Revealing bacterial biofilm community composition and its role in inducing metamorphosis of the tubeworm *Hydroides elegans*. Integrative and Comparative Biology 66(4): 640.

Zardus, J. D. and Hadfield, M. G. 2005. Genetic comparisons between native Atlantic and invasive Pacific populations of the barnacle *Chthamalus proteus*. Comparative and Integrative Biology 44(6): 671.

von Dassow, M. 2003. Development of a fluid transport system: An example from colonial organisms. Integrative and Comparative Biology 43(6): 817

vonDassow, M. 2004 Does fluid flow control pattern formation in a colonial suspension feeder? Integrative and Comparative Biology 44 (6): 658-658.

vonDassow, M. 2006. Influences of flow and feeding on colony organization in a bryozoan Talk presented at the Annual Meeting of the Society for Integrative and Comparative Biology, Orlando, FL.

Awards/Honors

Koehl, M. A. R. was awarded the Virginia G. and Robert E. Gill Chair by the University of California at Berkeley, 2005

Koehl, M . A. R. was one of the scientists featured in the series of children's books produced by the National Academy of Sciences: "Women's Adventures in Science". The book about Koehl's research, including work on larval settlement, is:
Parks, D. (2005) Nature's Machines. Joseph Henry Press, and Scholastic .

VonDassow, M. won the Adrian Wenner Award for the best student talk presented at the annual meeting of the Society for Integrative and Comparative Biology, 2004,